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# Use of Environmentally Enriched Housing for Rats with Spinal Cord Injury: The Need for Standardization

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**A variety of rehabilitation methods that increase social interaction and locomotor activity are reported to yield positive benefits in humans and animals with spinal cord injury (SCI). Environmental enrichment often incorporates group housing, increased cage size, and objects to increase social interaction and stimulate locomotor activity of animals. Others have reported that adult rats housed in enriched environments immediately after moderate contusion thoracic SCI show improvements in locomotion, but not in neurotransmission through or anatomy at the SCI site. In the present study, in contrast to previous reports, environmental enrichment did not improve the locomotion of rats with contusion thoracic SCI. Furthermore, as in previous reports, improvements were not observed for either electrophysiologic measures of neurotransmission through (transcranial magnetic motor-evoked potentials) and caudal to (magnetic-evoked interlimb reflex) the injury site or the amount of spared white matter at the epicenter. Determining the effectiveness of environmental enrichment to improve locomotor recovery in the SCI model requires standardization of housing procedures, outcome measures, and analyses.**

**Abbreviations:** SCI, spinal cord injury; BBB, Basso-Beattie-Bresnahan open-field locomotor scale; tcMMEP, transcranial magnetic motor-evoked potential; MILR, magnetic-evoked interlimb reflex

Rehabilitation methods that increase social interaction and locomotor activity have been used to improve locomotor recovery in humans and animals with brain and spinal cord injuries (SCI).[12,16,52,64] Specifically, environmentally enriched housing has shown beneficial effects in a variety of injury models. Environmentally enriched housing involves placing multiple animals together (that is, increasing housing density) and adding objects inside the cage (for climbing, tunneling, and so forth) to increase social interaction and locomotor activity. In addition to its usefulness in establishing optimal laboratory animal housing conditions (for example, social interaction and activity),[4,45,56] environmental enrichment has been studied for its potential beneficial physiologic effects on processes ranging from developmental growth[24] to aging.[39]

Environmental enrichment has been shown to promote recovery in a variety of rat brain injury models, including ischemic stroke[7,10,11,23,28,52,58] and trauma.[21,47,61] Beneficial effects include cognitive improvement or attenuation of the declination of cognitive function that occurs after brain injuries[7,11,30,51,61] as well as enhanced neurologic and motor function.[21,47] Modest improvements in the spontaneous recovery of open-field locomotor[31,59] and sensorimotor function[29,31,59] after contusion thoracic SCI, as well as attenuation of deficits postgrafting at this site,[44] have been noted in rats in environmentally enriched housing compared with pair-[29,31,59] or single-[44] housed control animals.

The underlying neuronal changes contributing to recovery after environmentally enriched housing are not yet clear. Neurogenic responses, such as changes associated with neuronal cell counts, size, length, and axonal processes, may play a role in the recovery process.[50] A corresponding recovery of function has been shown, with increased neurotrophic responses,[43] dendritic branching,[23] neurotrophins,[50,58] neuronal plasticity and growth,[27,28,30,39] and neuroprotection.[21,25,52]

Motor, propriospinal, and sensory axons in the spinal cord white matter[34,35,37,38,55] and thoracolumbar spinal cord neurons[19,37,38,53] contribute markedly to adult rat locomotor and sensorimotor functions. Neurologic changes involving this neural circuitry that may promote recovery of these functions can be assessed with behavioral, electrophysiologic, and morphologic techniques.

A study[31] using a standardized contusion thoracic SCI model[18] and locomotor function assessment[2,3] reported modest improvements in the spontaneous recovery of open-field locomotor function in environmentally enriched-housed rats. However, electrophysiologic and morphologic techniques revealed no effects on neurotransmission through descending rubrospinal tract axons or spared white matter at the SCI site. The transcranial magnetic motor-evoked potential (tcMMEP) technique is used to assess neurotransmission through thoracic and lumbar spinal cord ventrolateral white matter myelinated axons.[8,33,34,37,38] The magnetic-evoked interlimb reflex (MILR) technique assesses neurotransmission within the thoracolumbar spinal cord.[14] In the present study we sought to use electrophysiologic (tcMMEP, MILR) and morphologic (spared white matter) measures to evaluate the roles that the thoracolumbar neural circuitry play in the improved spontaneous recovery of open-field locomotor function previously[31,59] reported for rats in environmentally enriched housing after thoracic contusion SCI.

## Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Louisville and were performed according to standards used to support accredi-

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tation by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

**SCI and veterinary care.** We purchased 16 adult female Sprague-Dawley (Hsd:SD) rats from a commercial vendor (Harlan, Indianapolis, IN). The rats were confirmed specific-pathogen free via quarterly surveillance for rat coronavirus/sialodacryoadenitis virus, Sendai virus, pneumonia virus of mice, rat parvoviruses, rat minute virus, Kilham rat virus, Toolan H-1 virus, Theiler murine encephalomyelitis virus, reovirus type 3, lymphocytic choriomeningitis virus, mouse adenovirus strain 1, Hantaan virus, *Mycoplasma pulmonis*, cilia-associated respiratory bacillus, *Clostridium piliforme*, *Salmonella* spp, *Encephalitozoon cuniculi*, *Syphacia muris*, *Hymenolepis* spp, and *Giardia muris*. The rats (213.7 ± 10.0 g) were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). A moderate 12.5 g × cm contusion SCI was induced, as previously described,[3,38] at the T9 vertebral level of all rats by using the NYU/MASCIS Impactor (WM Keck Center for Collaborative Neuroscience, Piscataway, NJ).[18] After SCI, muscle and skin incisions were closed with silk sutures (4-0 Ethicon, Johnson and Johnson, Piscataway, NJ) and wound clips (Clay Adams, Sparks, MD), respectively, and Bacitracin Zinc Ointment USP (Fougera, Melville, NY) was applied to the sutured skin; 10 ml 5% dextrose (Sigma, St Louis, MO) in Lactated Ringer's solution (Braun Medical, Irvine, CA) was injected subcutaneously as needed. Each rat was returned to its cage with clean bedding. One half of the cage was placed on an electric heating pad for 1 d. Gentamicin (0.1 ml, 50 mg/ml intramuscularly, Schering-Plough Animal Health, Kenilworth, NJ), a broad-spectrum antibiotic, was administered at 2-d intervals for 6 d after SCI as prophylaxis against urinary tract infections until lower motor bladder control was restored. The Institutional Animal Care and Use Committee approved withholding analgesics based on the rationale that such treatments could influence neuroprotection (for example, opioids) and attenuate the natural secondary inflammatory cascade that occurs after SCI (for example, nonsteroidal anti-inflammatory drugs). Hydration and gastrointestinal function were monitored daily by assessing interscapular skin turgor and the overall amount of feces in the bedding, respectively. Bladders were emptied at least twice daily with gentle pressure on the lower ventral abdomen. Wound clips were removed 7 to 10 d after surgery.

**Housing.** All rats were housed singly in standard clear cages (44 × 24 × 20 cm) containing Alpha-dri bedding (Shepherd Specialty Papers, Watertown, TN) and rat chow (Purina Mills, Brentwood, MO) for 1 wk before SCI. A wire lid (Lab Products, Seaford, DE) held additional chow and a water bottle with a curved sipper tube spout and was covered with a plastic filter top. All rats were single- rather than pair- or group-housed prior to injury to eliminate the potential for interpretational confounds due to social interactions and changing housing conditions (paired or grouped before SCI to single after SCI). One day after SCI, the injured rats (n = 15; 1 rat died due to respiratory complications) were allocated randomly into 1 of the 2 housing conditions: environmental enrichment (n = 8, 4/cage) and control (n = 7). In a manner consistent with previous SCI studies,[29,31,59] rats in the environmentally enriched housing group were housed in a clear cage (61 × 43 × 20) containing Alpha-dri bedding (Shepherd Specialty Papers) and a plastic hiding hut (for ferrets), a polyvinyl chloride tube, a plastic climbing platform, 1 toy, and 4 wooden chews (Hartz, Secaucus, NJ; Figure 1). A wire lid held 2 water bottles with curved sipper tube spouts plus additional chow and was covered with a plastic filter top, as with control cage housing. Rats in the control group remained singly housed in standard cages. All cages were changed twice weekly. Daily handling and care by the animal veterinary care and study personnel were identical for both housing groups.

**Figure 1.** Environmentally enriched housing consisted of 4 rats in a cage that contained a variety of objects to encourage social interaction and locomotor activity. The wire lid and filter top were removed for the photograph.

**Behavior.** Locomotion was assessed as previously described[38] using the Basso-Beattie-Bresnahan (BBB) open-field locomotor scale.[2] Rats were evaluated individually by 2 experienced observers once before SCI and at weekly intervals after SCI for 12 wk.

**Electrophysiology.** tcMMEPs[33,38] were assessed once before SCI and at weeks 6 and 12 after SCI to evaluate descending neurotransmission through the injury site. A hand-held transducer coil (diameter, 5 cm) was positioned over the skull of each unanesthetized, lightly restrained rat to deliver a 70-ms magnetic stimulus pulse (MES-10, Cadwell Laboratories, Kenewick, WA). Electromyographic responses were recorded bilaterally using fine platinum wire electrodes inserted into the belly of the gastrocnemius muscles that are innervated by motoneurons in the L4–L6 spinal cord.[46] The ground electrode was placed at the base of the tail. A Sierra II data-acquisition system (MES-10, Cadwell Laboratories) was used to record and store the electromyographic waveform responses. MILR responses were assessed at weeks 6 and 12 after SCI to examine neurotransmission caudal to the injury site. The transducer coil was positioned over each hip, and electromyographic responses were recorded from the contralateral gastrocnemius muscles. The ground electrode was placed subdermally at the shoulder.

For both tcMMEP and MILR procedures, a stimulus intensity of 60% of maximal stimulator output was used. Each waveform was replicated to ensure reproducibility. The interstimulus interval was 30 s. Measures of all waveforms included the onset latency and peak-to-peak amplitude to examine the conduction along and caudal to the injured spinal cord, as previously described.[38] The onset latency was measured from the initiation of the stimulus artifact to the time of the first deflection from baseline. The peak-to-peak amplitude was measured as the distance between the waveform peak's highest and lowest points.

**Histology.** At 12 wk after SCI, all rats were deeply anesthetized with sodium pentobarbital (100 mg/kg intraperitoneally) and perfused intracardially with calcium-free Tyrode solution followed by 0.1 M phosphate buffer (pH 7.4; contains both sodium and potassium) containing 4% paraformaldehyde (Sigma, St Louis, MO). The T7–T11 spinal cords were removed, fixed overnight in the same solution, and stored for 3 d at 4 °C in

0.1 M phosphate buffer (as earlier) with 0.01% sodium azide (Sigma) containing 30% sucrose (Sigma). The spinal cord tissue was cut with a cryostat in serial, transverse 30-µm sections (3 sets), mounted onto charged microscope glass slides (Fisher Scientific, Pittsburgh, PA), and stored at –80 °C. Sections on 1 set of slides from each rat were rehydrated with double-distilled water, stained 5 to 8 min with 0.5% cresyl violet acetate (Sigma) solution,[37] rinsed with double-distilled water, dehydrated in a graded series of ethanol (AAPER Alcohol and Chemical, Shelbyville, KY), cleared in 100% xylene (Fisher Scientific), and covered with a coverslip (Fisher Scientific).

**Morphometry.** The cresyl violet-stained sections from each animal were photographed with an Optronics 3-chip cooled CCD digital video camera (Goleta, CA) attached to a Macintosh 9600/300 computer (Apple, Carpeteria, CA) using a Scion (Frederick, MD) CG-7 digitizer. The areas of spinal cord white matter were determined[37] by tracing every third image using a Wacom Intuos (Vancouver, WA) drawing tablet and Appleworks (version 6.0.4, Apple) and then saved as bit-map files. Thus, each image represented 90 µm of the T7–T11 spinal cord. The tracings were opened with *Image* (NIH, Bethesda, MD), and the areas of spinal cord white matter were calculated for each section. The extrapolated area of white matter that would normally lie between the rostral and caudal extents of the injury, indicated by the decline of white matter area and return to a plateau level, respectively, was determined. The percentage of white matter spared at the SCI epicenter then was calculated as the area of white matter at the SCI epicenter divided by the extrapolated area × 100.

**Statistical analysis.** All possible group comparisons of BBB left- and right-side scores at all testing times could not be performed due to degrees of freedom limitations and the resulting increase in the probability of a type I error that occurs with multiple *t* tests, respectively.[20] Therefore, paired *t* test comparisons were limited to pairs with the greatest likelihood of significance (that is, largest differences and smallest standard deviations) and averaged after determination of no significant difference. Changes in BBB locomotor scores over the test weeks were analyzed using repeated-measures analysis of variance with the between-groups factor. Because there would be insufficient degrees of freedom for analysis of all 12 test weeks in the repeated-measures analysis of variance, the early, middle, and late test weeks (that is, 1, 2, 6, 7, 11, and 12) were determined a priori for analysis.[20,40] Left- and right-side MILR responses were compared using paired *t* tests and did not differ. Averaged MILR response onset latency and peak-to-peak amplitude measurements were compared using repeated-measures analysis of variance with the between-groups factor (degrees of freedom were adjusted to correct for unequal variances when appropriate).[20] Post hoc *t* tests (Tukey HSD or Student Newman-Keuls, as appropriate) were performed after determination of significant repeated-measures analysis of variance main effects.[20] The percentage of white matter spared at 12 wk post-SCI was compared between groups by using independent *t* tests. The distribution of the data showed a separation into low (less than 20%) and high (20% to 40%) percentages by group. Therefore, the frequency of rats within the enriched and control groups with lower and higher percentages was compared using Fisher exact chi square test. Data analyses were performed using a statistical software package (SPSS version 10.0, SPSS, Chicago, IL). Values are expressed as mean ± 1 standard deviation, and the threshold for statistical significance was $P = 0.05$.

## Results

BBB scores were examined over 12 wk after SCI to detect improvement in characteristics associated with open-field, over-ground locomotor function.

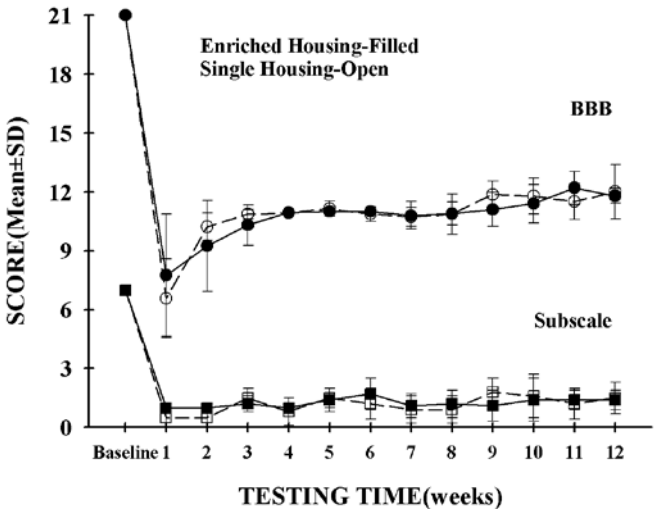


**Figure 2.** Open-field locomotion of rats in the environmentally enriched (n = 8) and control (n = 7) singly housed groups was impaired similarly at 1 wk post-SCI and then spontaneously recovered (albeit not to baseline levels) thereafter. At 1 wk post-SCI, most rats in both groups were unable to stand or support their weight. By 12 wk post-SCI, all rats were able to walk with some coordinated stepping. BBB scores (mean ± 1 standard deviation) assessing locomotor recovery or BBB subscale scores derived from components of the BBB scoring method (mean ± 1 standard deviation) did not differ significantly between the groups over the 12 wk post-SCI.

**Behavior.** The mean pre-SCI left- and right-side BBB scores for each rat were 21. Left- and right-side post-SCI scores did not differ from one another and were averaged (Enriched: t = 1.5, df = 7; Control: t = 2.3, df = 6; $P > 0.05$). Most animals in both groups were unable to stand or support their weight (BBB < 9) at week 1 after SCI (Figure 2). The BBB scores did not differ between groups at this or any other time (F = 0.07; df = 1, 13; $P > 0.05$). BBB scores of both groups increased significantly over the testing period (F = 31.4; df = 1.8, 65; $P < 0.001$). A significant interaction between the group and week factors was not detected, indicating a similar pattern of changes in the BBB scores for the 2 groups over the test weeks (F = 1.4; df = 1.8, 65; $P > 0.05$). BBB scores of the enriched group were significantly higher at week 11 (12.2 ± 0.8) compared with week 1 after SCI (7.8 ± 3.1; F = 12.8, df = 1.4, 10; $P < 0.005$; post hoc *t* tests: t = 4.3; df = 6, 10; $P < 0.05$). BBB scores of the control rats were also higher at week 11 (11.5 ± 0.9) compared with week 1 (6.6 + 2.0; F = 22.0; df = 2.2, 13; $P < .001$; post hoc *t* tests: t = 3.6; df = 5, 13; $P \leq 0.01$). At study termination (week 12), the rats could walk with some coordinated stepping.

Two previous studies used nonparametric statistical analyses to compare locomotor BBB scores and BBB subscale scores between housing groups and found significant differences.[31,59] The BBB subscale score is a measure that assigns points to some components of walking characteristics assessed during the BBB test, including toe clearance, paw position, trunk instability, and tail position.[32] In the current study, average BBB subscale scores for both groups were low (0.5 to 1.8) throughout the testing period (Figure 2). To facilitate comparison, we used the same nonparametric statistics of the cited studies to compare the BBB and BBB subscale scores of the housing groups in the current study. No significant differences were found between the housing groups, although the averages and standard deviations were similar to those reported in the previous studies. Therefore, data from the present study indicate that spontaneous recovery of locomotor function occurred in both groups after

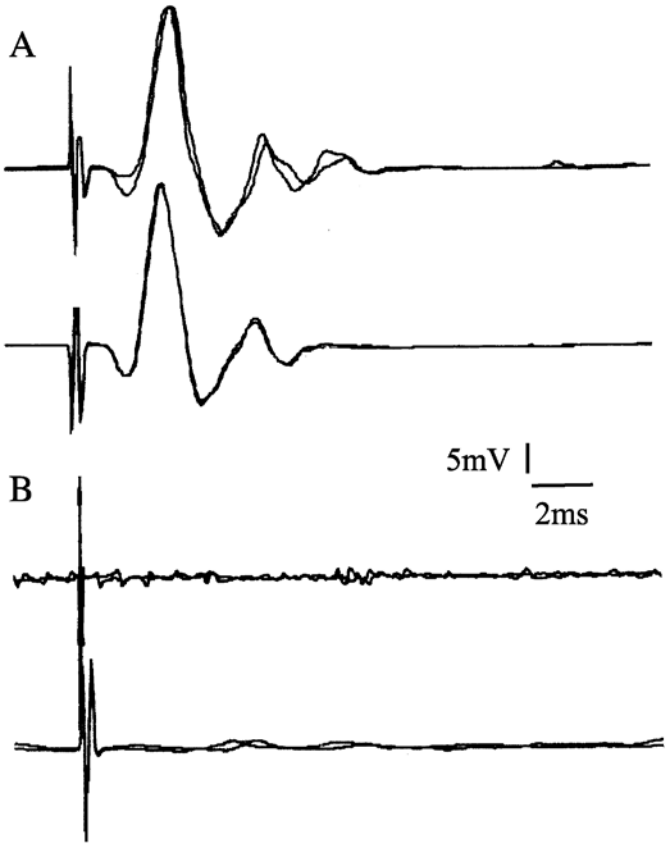


**Figure 3.** MILR and tcMMEP procedures, 2 highly objective and reliable electrophysiologic methods, were used to assess neurotransmission caudal to and through, respectively, the thoracic SCI site. (A) Representative examples of MILR response waveforms recorded from the gastrocnemius muscles after magnetic stimulation of the hip from rats (enriched group) at 6 (upper) and 12 (lower) wk post-SCI. (B) These representative examples illustrate the lack of measurable tcMMEP responses at 6 (upper) and 12 (lower) wk post-SCI from the enriched-housed group of rats. Vertical bar, 5 mV; horizontal bar, 2 ms.

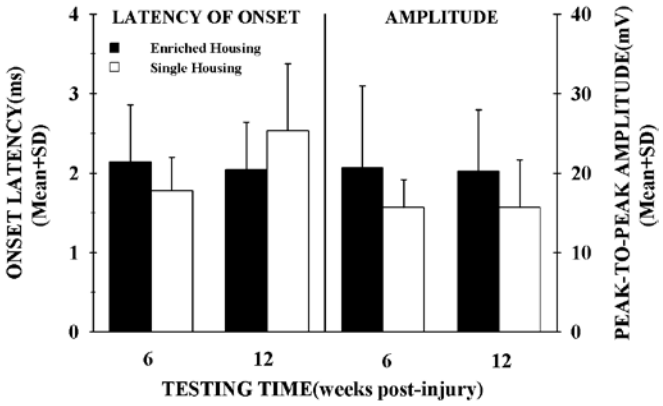


**Figure 4.** MILR responses are a measure of neurotransmission caudal to the injury site. The waveform latency of response onset time and the peak-to-peak amplitude of the response of the rats were measured post-SCI. Both the average onset latency (ms, left) and peak-to-peak amplitude (mV, right) measures of the MILR responses (mean + 1 standard deviation) at weeks 6 and 12 post-SCI were similar between rats housed in an enriched environment (n = 8) and control single-housed animals (n = 7).

SCI and that environmentally enriched housing did not further improve recovery.

Descending and ascending motor, propriospinal, and sensory axons within the thoracic and lumbar spinal cord ventrolateral white matter[8,34,35,37,38,55] as well as neurons in the thoracolumbar spinal cord[19,37,38,53] are critical for adult rat locomotion.[34,35,37,38] We used 2 highly objective and reliable electrophysiologic methods to assess neurotransmission of this spinal cord circuitry at 6 and 12 wk after SCI.

**Electrophysiology.** As illustrated in Figure 3 A, MILR responses were present in all rats at 6 and 12 wk after SCI. The onset latencies did not differ between groups or between weeks 6 and 12 after SCI (Group: $F = 0.06$; $df = 1, 13$; $P > 0.05$; Week: $F = 2.05$; $df = 1, 13$; $P > 0.05$; Figure 4, left). Response amplitudes (Figure 4, right) also were similar between the groups at both test weeks (Group: $F = 3.7$; $df = 1, 13$; $P > 0.05$; Week: $F = 0.95$; $df = 1, 13$; $P > 0.05$). These data indicate that environmentally enriched housing did not alter neurotransmission caudal to the SCI.

For normal tcMMEP responses, onset latency is 5 to 6 ms and peak-to-peak amplitude is 15 to 25 mV.[33] Consistent with previous reports of rats with similar injuries,[38] we did not obtain measurable tcMMEPs from the gastrocnemius muscles of any control rats or any enriched housing rats at 6 and 12 wk after SCI (Figure 3 B). These data indicate that reproducible disruption of conduction through myelinated axons in the ventrolateral white matter[8,34,35,38] occurred after thoracic contusion SCI and was not altered by housing condition. Both groups of rats had similar BBB scores at these time points (Figure 2).

Compared to that of normal spinal cords,[34,38] the white matter in the thoracic spinal cords of the control and enriched housing rats was extensively damaged after contusion SCI, as previously reported for rats with similar injuries.[3,38] The white matter at the epicenter of the thoracic SCI of each rat was measured to determine whether this amount differed between housing groups, and whether this difference was associated with the behavioral and electrophysiologic measures. The percentage of white matter spared at the SCI epicenter equaled the area of white matter at the SCI epicenter divided by the extrapolated area × 100 (Figure 5, inset).

**Morphometry.** Comparison between the 2 groups did not reveal a significant difference in the percentage of total white matter spared at the epicenter in the rats housed in the enriched and control environments (24.5% ± 9.5% and 16.5% ± 7.4%, respectively; $t = 2.2$, $df = 13$, $P > 0.05$). The distribution of the data was bimodal, separating into categories of low (less than 20%) and high (20% to 40%) percentages of spared white matter. Further, 5 of the 8 (71.4%) rats in the enriched group had a high percentage of spared white matter, compared with 2 of 7 (28.6%) in the control group, but these proportions were not significantly different ($\chi^2 = 1.7$, $df = 1$, $P > 0.05$). White matter sparing was not related to MILR measures or BBB scores at week 12.

## Discussion

The present study did not detect significant differences in locomotor, electrophysiologic or morphometric outcome measures between environmentally enriched- and control-housed rats with moderate thoracic SCI. The behavioral results were unexpected in light of findings in 2 studies[31,59] that used a similar contusion SCI model and found significant improvement in locomotor BBB and subscale scores after environmentally enriched housing, as did other studies that used compression[12] and laceration[65] SCI. However, our results were similar to those of a comparable study[13] that used female Sprague-Dawley rats (200 to 300 g), the same contusion SCI model, and various housing paradigms.

The present study differed in several ways from previous investigations[31,59] that reported locomotor differences between housing groups. Differences include rat strain, gender, injury

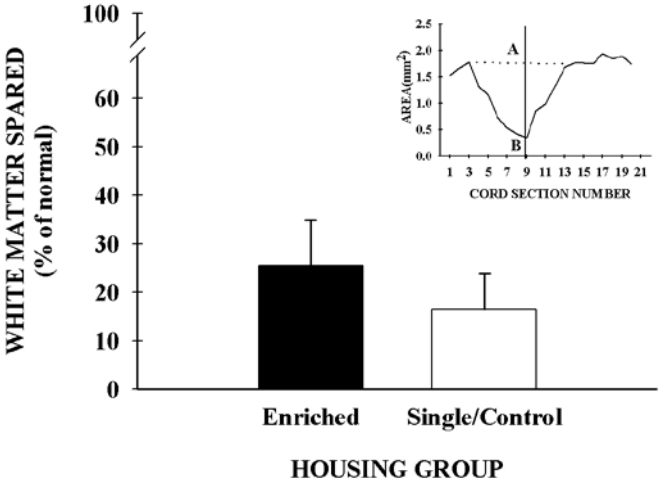


**Figure 5.** Average (+ 1 standard deviation) percentage of white matter spared at the spinal cord epicenter of rats in the environmentally enriched housing (n = 8) and control single-housed (n = 7) groups was not significantly different. The inset shows measurements of the amount of white matter spared (*y* axis) across sections from rostral to caudal (*x* axis) of a representative injured cord. The extrapolated areas of white matter (A) that would normally lie between the rostral and caudal extents of the injury (dotted line) and at (solid vertical line) the epicenter (B), where the amount of spared white matter decreased to its lowest point, are shown.

level, study duration, onset of enrichment, animal density, cage size, and available cage space per animal (Table 1). Female Sprague-Dawley and Wistar rats have similar locomotor and sensorimotor walking characteristics.[62] Assessment with the BBB open-field locomotor scale has shown that the locomotor recovery of male Sprague-Dawley and Wistar rats was not different at 5 wk after a SCI similar to that used in the present study.[42] Moreover, the BBB scores and subscale scores of the female Sprague-Dawley control, singly housed rats (213.7 ± 10.0 g) in the present study with a T9 injury and those of female (200 to 225 g; T9 and T8 SCI)[30,59] and male (253 ± 4.2 g; T10 SCI)[28] Wistar control, pair-housed rats were similar (Table 1). The BBB scores of female Sprague-Dawley control, singly housed rats (250 g) with a T9 injury in the new study[13] also were similar to those of the present study.

The duration of the present study differed from that of other studies,[29,31,59] but the average BBB and BBB subscale scores of the control and enriched housing groups were similar at 3 to 4 wk after SCI and at the time of study termination (Table 1). The effect of different times of initiation of environmentally enriched housing in the present (1 d after SCI) and previous investigations (Table 1) is uncertain. BBB and BBB subscale scores increased in female Wistar rats placed in environmentally enriched housing immediately after contusion SCI[31,59] but not after a 3-wk delay.[29] Other studies yielded conflicting information. As compared with rats with enriched housing throughout one study, further improvement in BBB scores did not occur in group-housed injured rats placed in enriched housing immediately after SCI, and BBB scores were not affected by removing environmental enrichment immediately after SCI.[13] In a preliminary study, female Sprague-Dawley rats showed enhanced locomotor activity when environmentally enriched housing was delayed for 3 mo after moderate to severe contusion thoracic SCI[15] and when environmentally enriched housing was combined with neural transplantation at 7 mo after laceration thoracic SCI.[65] Moreover, female Fischer-344 rats that received Schwann cell or olfactory ensheathing glial cell transplants immediately after thoracic transection SCI and were placed in environmentally enriched housing 1 wk later showed no deterioration of BBB scores compared with transplant rats that remained singly housed.[44]

Guidelines have been established to ensure that rats receive at least a minimal amount of cage floor space, [45] although optimum housing density and animal preference may vary depending on the experimental conditions and manipulations. Cage size, animal density, and available cage floor space per animal can all influence behavior.[43,48] Normal adult rats preferred a larger cage when given the opportunity to work for access to the cage of choice.[48] Moreover, locomotor activity in cages of singly housed rats fell with a decrease in cage size and remained unchanged without a change in cage size.[43] The proportion of the minimal required floor space available to the singly housed control rats in the present study was more than twice that of pair-housed control rats in the 3 previous studies using similar injuries,[29,31,59] but the average locomotor BBB scores of these animals were not different (Table 1). Because locomotor activity in the cage was not measured in these studies, conclusions about its effect on the data cannot be ascertained. Studies that assess locomotor activity in the cage are necessary to address this issue.

Increased social interaction may provide a stronger influence on rats than cage size.[1,9,57] For example, the locomotion of normal adult rats exploring in an open-field increased when their housing group size increased;[1] the cage space available to each group of rats was equivalent. Even though each environmentally enriched cage in the present study contained 4 rats with nearly 4 times the minimal required floor space, their locomotor BBB and subscale scores were not different from those of the singly and pair-housed control rats of the present and previous studies.[29,31,59] Female Sprague-Dawley rats housed in groups of 4 with enriched environments immediately after the same injuries as in our rats had locomotor BBB scores similar to those of rats housed in groups of 4 without enriched environments and rats housed singly in standard cages.[13] In contrast, 2 previous studies using acute treatment[31,59] found that the BBB scores of the 8 to 15 rats housed in environmentally enriched enclosures were significantly higher than those of pair-housed control rats. In those studies,[31,59] each rat in the environmentally enriched housing had similar proportions of the minimum required floor space as did the rats in the present study (Table 1), yet in the previous studies, the locomotor BBB scores at week 8 after SCI were, on average, 1 point greater. Collectively, these data suggest that a critical level of social interaction, and perhaps locomotor activity, must be reached in an enriched environment before physiologic changes lead to enhanced locomotor recovery.

Because relationships among animal density, cage size, social interaction, and locomotor activity are variable and complex, systematic investigations are needed to standardize the environmental enrichment technique in conjunction with SCI models for future assessment of its beneficial effects.[5,6,17] To allow comparison among SCI studies, standardization and refinement of SCI models,[3,18,26,54] environmental housing parameters[5,6] and experimental protocols is essential. Moreover, enriched cages have contained objects such as cylindrical tubes, arched tunnels, huts, running wheels, and climbing platforms. Because various cage conditions may not be equivalent,[5] identification and standardization of specific manipulata is vital.[4,5,6,17,36]

SCI studies with environmental enrichment have used different statistical analyses (that is, parametric;[13,29] nonparametric[31,59] and the present study) to compare the locomotor BBB scores and subscale scores between housing groups. Some studies[31,59] found significant effects of housing by using nonparametric rankings of locomotor scores. Nonparametric analyses of BBB and BBB subscale scores in the present study revealed no significant differences between the housing groups, although

**Table 1.** Experimental parameters and results of environmental enrichment studies using contusion spinal cord injury (SCI) models

| | Present study | Koopmans et al.[29] | van Meeteren et al.[59] | Lankhorst et al.[31] |
|---|---|---|---|---|
| Animals and injury type | | | | |
| Gender | Female | Male | Female | Female |
| Strain | Sprague-Dawley | Wistar | Wistar | Wistar |
| Injury level | T9 | T10 | T8 | T9 |
| Study termination point (weeks post-SCI) | 12 | 11 | 14 | 8 |
| Housing conditions | | | | |
| Rats/cage | | | | |
| Environmentally enriched | 4 | 9 | 8–12 | 15 |
| Control | 1 | 2 | 2 | 2 |
| Total sample size | | | | |
| Environmentally enriched | 8 | 9 | 8–12 | 15 |
| Control | 7 | 8 | 10 | 15 |
| Cage size (in.$^2$) | | | | |
| Environmentally enriched | 408 | 1891 | 1891 | 1891 |
| Control | 166 | 50 | 50 | 50 |
| Available floor space/rat (in.$^2$) | | | | |
| Environmentally enriched | 102 | 210 | 158–236 | 126 |
| Control | 166 | 25 | 25 | 25 |
| Proportion of minimal required floor space/rat (-fold) | | | | |
| Environmentally enriched | 3.5 | 8.2 | 5.4–7.2 | 4.3 |
| Control | 5.7 | 2.9 | 2.9 | 2.9 |
| Initiation of enriched housing post-SCI | 1 d | 3 wk | immediately | immediately |
| BBB results | | | | |
| Week 8 (mean ± 1 standard deviation) | | | | |
| Environmentally enriched | 10.9 ± 0.8 | 11.4 ± 1.0[a] | 12.1 ± 1.5[a] | 12.5 ± 0.9[a] |
| Control | 11.0 ± 1.2 | 11.3 ± 1.0[a] | 11.3 ± 0.6[a] | 11.3 ± 3.5[a] |
| Housing group differences[a] and type of analysis | | | | |
| BBB scale (0–21), week 8 | 0.1, P and NP | 0.1, P | 0.9[b], NP | 1.4[b], NP |
| BBB subscale (0–7), week 8 | 2.0, P and NP | 0.3, P | 1.5[b], NP | 1.6[b], NP |

NP, nonparametric analysis (used to compare housing groups' ranked scores); P, parametric analysis (used to compare housing groups' averaged scores).
[a]Group means, differences, and standard deviations were extrapolated from published graphs.
[b]Significant ($P < 0.05$) difference between housing groups.

the averages and standard deviations were similar to those of previous studies. Low housing density is reported to influence statistical power and effect size as well as outcome measures.[41] Small (1- to 1.5-point) differences in BBB scores between groups at the study termination were observed in the present and previous studies using different statistical analysis.[29,31,59] The similar BBB scores obtained for the environmentally enriched groups in the present and previous studies (Table 1) and the small-magnitude differences in the BBB scores that were significant with nonparametric[31,59] but not parametric statistics do not conclusively support enhancement of locomotor recovery associated with environmental enrichment after contusion thoracic SCI.

In addition to defining enhanced housing for SCI rats (for example, animal density, cage size, social interaction, activity, and enclosure manipulata), terminology specifying meaningful degrees of recovery of function after SCI is important. A change in outcome measures may not necessarily represent improvement.[5,6] The change in locomotor recovery that represents a significant functional improvement depends on the specific goals of the experiment. For example, a statistically significant 1-point difference in the last stage of recovery at the higher range of the BBB locomotor scale (15 to 21 points) represents a change in fine-motor control (for example, whether the paw is parallel or rotated relative to the body when walking). In comparison, a 1-point change from 8 to 9 and 9 to 10 in the middle range of the scale (8 to 14 points) represents the ability of a rat to stand and walk and is biologically significant. In addition, the return of a tcMMEP after SCI would indicate a biologically relevant change and improved neurotransmission through the damaged spinal cord, despite negligible functional improvement.[34]

This distinction, established using definable criteria, would be particularly relevant in the absence of compelling corroborating objective electrophysiologic or morphologic statistical differences between housing groups.[31,65] Until standards are established, the use of a locomotor task with more objective measurements than the BBB locomotor scale would improve comparability among studies. To that end, a new locomotor task based on gait analysis and coordination has been proposed.[29] Scores on this test were significantly higher with environmental enrichment compared with control housing, whereas significant differences were not detected using locomotor BBB scores.[29] A more sensitive measure also may prove useful in assessing the biological importance of improvements in gait and coordination.

The beneficial effects of environmentally enriched housing are based on complex, interrelated variables. [60] Whether environmentally enriched housing has positive effects on outcome measures depends on the circumstances.[4–6] The specific conditions under which enrichment improves behavioral or neuronal

outcome measures in SCI rats are not yet certain.[4,6,41,57] This effort is limited by the lack of standardization and need for refinement in environmentally enriched housing protocols.[5,56,63] Defining the cage structure, objects contained in the cage, social contact, cage space used, and animal density and controlling physiologic factors (for example, gender, strain, age, weight), and maintaining cost-effective and practical experimental procedures are essential for rat SCI models.[22,49,63]

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